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Pairing context determines condition-dependence of song rate in a monogamous passerine bird

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Condition-dependence of male ornaments is thought to provide honest signals on which females can base their sexual choice for genetic quality. Recent studies show that condition-dependence patterns can vary within populations. Although long-term association is thought to promote honest signalling, no study has explored the influence of pairing context on the condition-dependence of male ornaments. In this study, we assessed the influence of natural variation in body condition on song rate in zebra finches (*Taeniopygia guttata*) in three different situations: during short and long encounters with an unfamiliar female, and within heterosexual mated pairs. We found consistent individual differences in male directed and undirected song rate. Moreover, body condition had a positive effect on song rate in paired males. However, male song rate was not influenced by body condition during short or long encounters with unfamiliar females. Song rate appears to be an unreliable signal of condition to prospective females as even poor-condition birds can cheat and sing at a high rate. By contrast, paired females can reliably use song rate to assess their mate's body condition, and possibly the genetic quality. We propose that species' characteristics, such as mating system, should be systematically taken into account to generate relevant hypotheses about the evolution of condition-dependent male ornaments.

1. Introduction

Understanding the function of male ornaments is central to the study of sexual selection [1,2]. Indeed, sexual dimorphism is often assumed to be the result of selection through male–male competition or female mate choice [3]. Ornaments can either be used as weapons during male contests or be the target of female sexual choice, or both [4]. In particular, male ornaments can act as signals conveying different types of information to females [3]. For instance, only males of good genetic quality are expected to be able to develop and maintain costly ornaments under the handicap hypothesis [5,6]. Various costs can be associated with developing ornaments, such as the amount of time devoted to their maintenance [7], the energy expenditure associated with their expression [8] or the increased risk of being detected by predators [9]. Therefore, male ornaments, for which development and maintenance depends on individual condition, can be reliable signals of genetic quality that females can use to assess potential mates [10]. To verify this assumption, several studies have attempted to link the elaboration of male ornaments to individual body condition [11]. Body condition has thus been found to mediate the development of ornaments in several species [12,13], whereas some studies have shown that condition-dependence patterns may vary within the same populations [14–16].

In birds, male song is one of the most studied sex-specific activities in relation to mate choice and sexual selection [17], and is highly variable within populations

[18–20]. A high song rate is likely to be costly, in terms of either energy expenditure [8,21] or time lost for other activities [22]. Inter-individual variation in song rate is thus thought to reflect genetic variation in condition, and thus differences in genetic quality [10]. However, the phenotypic covariation between song rate and body condition varies across studies, and even within species [23,24]. In particular, although body condition is heritable [25] and plays a role in female preferences [26] in zebra finches (*Taeniopygia guttata*), results from successive studies conducted with this species are inconclusive [24]. For instance, De Kogel & Prijs [27] showed that males from enlarged broods, which typically suffer from lower structural size [28], had a lower song rate, whereas Tschirren *et al.* [29] found the opposite. By contrast, Bolund *et al.* [30] found no evidence for condition-dependence of male song rate.

However, the influence of social context on the condition-dependence of male ornaments has not been addressed so far. Zebra finches are socially monogamous with long-lasting pair-bonds [31]. Several lines of evidence suggest that pairing context may affect the extent to which females focus on male song rate in this species [32]. For instance, unpaired female zebra finches do not pay attention to the songs of unfamiliar males [33] (but see [34]), whereas mated females pay more attention to their mate's song compared with the song of an unfamiliar male [35]. Furthermore, investment into current reproduction is stimulated in females paired with males singing at a high rate [36]. Taken together, these studies suggest that the influence of male song on female behaviour could vary depending on pairing context. Yet nothing is known about the pattern of song rate's condition-dependence across pairing contexts. Specifically, to what extent long-term pair-bonding may promote honest signalling between reproductive partners has not been examined, though pair-bonding has been shown to promote cooperation in zebra finches [37]. However, a recent model indicates that long-term commitment in resource defence may promote honest signalling during agonistic interactions [38]. Here, we build on the same approach to predict that pair-bonding should promote honest signalling and generate a positive link between body condition and song rate. This is because, following the handicap principle [5], the costs of sustaining a high song rate over long timescales (as over the course of a pair-bond) are expected to be higher (and thus unaffordable) for poor-condition individuals compared with good-condition individuals. Conversely, encounters with unfamiliar females should motivate males of any condition to sing at similar rates. This investment remains affordable even for poor-condition individuals because of the limited duration of the interaction.

In this study, we investigate whether male song rate depends on natural variation in body condition in zebra finches in a controlled environment, and whether this relationship depends on pairing context. We first assessed natural variation in male body condition, and then quantified song rate in three different situations: during short and long encounters with an unfamiliar female, which enabled us to assess the effect of trial duration on signalling, and over the course of a sexual pair-bond with a female.

2. Methods

(a) Study subjects

The zebra finch is a small passerine bird species mainly found natively in Australia, widely used in mate-choice studies [24,39], and in which only males produce a developed song [24,31]. Ninety-one young adult males and 91 young adult females from the Université de Bourgogne's colony were used. Birds were kept indoors in unisex groups of two or three in a single experimental room, allowing auditory and visual contacts between individuals. Birds were maintained in home cages (l × w × h: 60 × 33 × 30 cm), containing four perches and four feeders. Room temperature was maintained at 22 ± 2°C, and the photoperiod was 13 L: 11 D cycle. Each individual was identified by an orange-numbered ring (A.C. Hughes, Hampton Hill, UK; size XF). In addition, 12 adult males and 12 adult females from the University of Exeter's colony were used. Outside experiments, these birds were kept indoors in unisex groups of two to five birds in a single experimental room and maintained in home cages (l × w × h: 100 × 50 × 50 cm), containing four perches and four feeders. Room temperature was maintained at 19 ± 1°C, and the photoperiod was 14.5 L : 9.5 D cycle. Each individual was identified by an aluminium numbered ring.

All individuals had undergone mate-choice trials during a previous unpublished study, thus avoiding the problem that females are less selective or less responsive when courted for the first time in their life [33]. Birds were provisioned with millet seeds, cuttlebones and water ad libitum. After the experiments, birds were placed back in their home cages to be used in subsequent studies.

(b) Assessment of body condition

Between 4 and 12 days prior to the experiments, male tarsus length was measured twice with a calliper to the nearest 0.1 mm, and each male was weighed twice to the nearest 0.01 g with an electronic balance (Ohaus, Scout Pro SPU202). Both measures were taken by M.D. on two different days (range of the difference between days of measurements: 1–7), but at the same time of day for each and every bird. This procedure allowed us to assess measures' repeatability. Then, the average of tarsus length and weight was calculated for each individual and used thereafter. Two indices of body condition were computed. First, residual body mass was estimated for each individual from the residuals of an ordinary least-squares regression of $\log_{10}$(weight) on $\log_{10}$(tarsus length) [40], plotted from the entire sample ($R^2 = 0.11$, $F_{1,102} = 12.4$, $p < 0.001$). Second, we computed the scaled mass index following Peig & Green [41,42], which, unlike residual body mass, does not assume isometry (i.e. body composition not changing with size). By contrast, the scaled mass index allows allometry (i.e. body composition potentially changing with size) by including a scaling exponent in its calculation [41]. Both indices were found to be highly correlated (Pearson's $r_{101} = 0.94$, 95% confidence interval (CI) [0.91;0.95], $p < 0.0001$; figure 1). Moreover, results obtained with either residual body mass or scaled mass index as predictors in subsequent models proved to be substantially similar. Thus, we hereafter present results obtained only with the scaled mass index as a predictor.

(c) Short-encounter trials

Fifty-one males from the Université de Bourgogne's colony underwent brief encounters with 51 different unfamiliar females from the same colony. To this end, one female was placed in an empty home cage. Then, an unfamiliar male was introduced into the same cage. The interaction between the two individuals was video-recorded for 5 min [33], before the birds were placed back in their respective home cages. This procedure, also called 'no-choice trial', is commonly used for the assessment of female preferences or receptivity in zebra finches and other animals [33,43–45]. Each male performed two short-encounter trials on the same day with the same female, with one trial in the morning and one trial in the afternoon. Behavioural trials

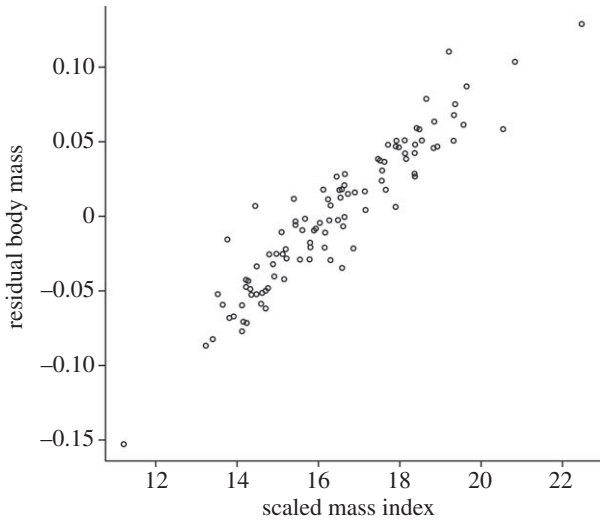

Figure 1. Relationship between residual body mass and scaled mass index, two computations of body condition.

were recorded with a video-camera (JVC Everio GZ-MG20) placed 50 cm in front of the cage. Male zebra finches produce two different types of song: directed song (DS), which is a sexual display and corresponds to the male standing up in front of the female, facing her and singing; and undirected song (US), which is not directed to the female and corresponds to any other occurrence of song [24,31]. Both are easily distinguishable and are rarely performed together during the same song bout. The duration of DS was measured using a stopwatch. Males did not perform any US during these trials.

(d) Long-encounter trials

Twelve males from the University of Exeter's colony underwent long encounters with 12 different unfamiliar females from the same colony. The procedure was exactly the same as in the short-encounter experiments, except that males were kept in unisex groups of two for 6 days before trials. Then, one of them was removed from the cage (l × w × h: 50 × 50 × 50 cm), and an unfamiliar female was introduced for 1 h in the morning and again for 1 h in the afternoon. Cages were always provided with water and millet seeds. These trials allowed us to record both male DS and US duration over a longer period of time than for the short-encounter trials. Therefore, we could investigate the effect of trial duration on male display towards an unfamiliar potential mate. Furthermore, this enabled a direct comparison with the procedure used in subsequent within-pair observations.

(e) Within-pair observations

Forty females from the Université de Bourgogne's colony were used. Each was non-randomly paired to either a male they had preferred during a 90 min mate-choice trial using a standard two-way apparatus, or to the non-preferred male, both coming from the Université de Bourgogne's colony [46]. This procedure enabled us to test for the effect of male preferred or non-preferred status on singing activity for the purpose of another study. All the birds used in this treatment were different from those used in the short-encounter trials. The newly formed pairs were kept in home cages for 6 days. On the following day, they were video-recorded twice, for 1 h in the morning and 1 h in the afternoon. We measured DS and US duration from the video footage using a stopwatch. In addition, we checked that pair members effectively formed a sexual pair by recording the occurrence of affiliative behaviours, such as clumping or allopreening.

(f) Statistical analyses

For each male and each trial, we computed a DS rate (s min^{-1}), a US rate (s min^{-1}) and an all-song (AS) rate from the sum of the amount of time spent singing DS and US. To this end, we divided the recorded duration of singing by the duration of the corresponding trial. Yet, in all subsequent models, the amount of time spent singing was used as the dependent variable (except for descriptive analyses) as rates had highly heterogeneous variances between treatments, unlike time spent singing [47]. As we did not compare directly the amount of time spent singing between treatments but rather its interaction with body condition or time of day, the scale of the dependent variable used was not important. Using time spent singing was thus appropriate here. In any other cases, amounts of time spent singing were compared within a single treatment, making the question of the scale used also redundant. Indeed, amounts of time spent singing were here proportional to the rates.

Consistency of time spent singing was assessed separately for each song type and each treatment. We used the 'rptR' package [48] to estimate individual repeatability in time spent singing. Variables were log- or square-root-transformed when possible. Intra-class correlation coefficients (R) were then estimated using models fitted with Gaussian distributions, along with their 95% CI and p-value [49]. We did not fit a Poisson distribution to the model because of a huge estimated overdispersion of the residuals. As the distribution of time spent singing US in the long-encounter treatment did not reach normality even after transformation, we still fitted a Poisson distribution to estimate its repeatability [48].

The joint influence of body condition, time of day (morning or afternoon) and treatment (all added as predictors) on time spent singing was assessed separately for each song type, using generalized linear mixed models fitted with Poisson distributions. Male identity was added as a random intercept factor. Moreover, following the recommendation of Schielzeth & Forstmeier [50], time of day was placed as a random slope factor. Post hoc analyses were conducted to determine differences between pairs of treatments in the effect of body condition on time spent singing, with the interaction between body condition and treatment as a predictor and male identity as a random intercept factor. Moreover, we investigated the effect of body condition on time spent singing within treatments using linear mixed models with male identity as a random intercept factor and body condition as a predictor. We provided Pearson's (r) or Spearman's (r_S) correlation coefficients as effects sizes of the influence of body condition on time spent singing, with each male appearing twice (morning and afternoon value) in the analysis. However, we were unable to report the corresponding 95% CI as they prove to be unreliable when computed from mixed models (S. Nakagawa 2012, personal communication). Daily variation in time spent singing was computed as Cohen's d [49,51] between morning and afternoon trials for each treatment and each song type and its corresponding 95% CI [49]. Analyses were performed with JMP v. 5.0.1. statistical software (SAS Institute, Cary, NC, USA) and the statistical freeware R v. 2.11.1 and 2.13.0 [52]. Data from this study have been deposited in Dryad (doi:10.5061/dryad.b191j).

3. Results

(a) Descriptive analyses

By testing each treatment's sample separately, all morphometric measurements were significantly repeatable (within-pair observations: tarsus length: Pearson's $r_{38} = 0.70$, 95% CI [0.50;0.83], $p < 0.0001$; weight: $r_{38} = 0.98$, 95% CI [0.97;0.99], $p < 0.0001$; short-encounter trials: tarsus length: $r_{48} = 0.77$, 95% CI

[0.63;0.86], $p < 0.0001$; weight: $r_{48} = 0.95$, 95% CI [0.91;0.97], $p < 0.0001$; long-encounter trials: tarsus length: $r_{10} = 0.67$, 95% CI [0.16;0.90], $p < 0.017$; weight: $r_{10} = 0.99$, 95% CI [0.95;0.99], $p < 0.0001$). Mean tarsus length did not vary between the two colonies ($F_{1,101} = 0.51$, $p = 0.47$). Similarly, mean body condition did not vary between the two colonies ($F_{2,100} = 0.66$, $p = 0.42$), nor among treatments ($F_{2,100} = 0.63$, $p = 0.53$). In the within-pair treatment, preferred and non-preferred males did not differ in any of the recorded variables (body condition: $F_{1,38} < 0.01$, $p = 0.94$; DS rate: $\chi^2 = 0.04$, d.f. = 1, $p = 0.84$; US rate: $\chi^2 = 2.22$, d.f. = 1, $p = 0.14$; AS rate: $\chi^2 = 1.95$, d.f. = 1, $p = 0.16$). US rate was significantly related to DS rate in both the within-pair treatment ($\chi^2 = 190.2$, d.f. = 1, $p < 0.0001$, Pearson's $r_{78} = 0.42$) and the long-encounter treatment ($\chi^2 = 105.4$, d.f. = 1, $p < 0.0001$, Spearman's $r_S = 0.14$). Yet the intensity of the relationship between DS rate and US rate differed between the within-pair and the long-encounter treatments (interaction DS rate × treatment: $\chi^2 = 5.07$, d.f. = 1, $p = 0.024$).

(b) Daily variation of time spent singing

Time spent singing DS was influenced by time of day in interaction with treatment (interaction time of day × treatment: $\chi^2 = 25.8$, d.f. = 2, $p < 0.0001$). Post hoc analyses revealed that time spent singing DS significantly decreased from morning to afternoon in the long-encounter treatment ($\chi^2 = 14.2$, d.f. = 1, $p < 0.001$), but not in the within-pair ($\chi^2 = 0.58$, d.f. = 1, $p = 0.45$) or the short-encounter treatments ($\chi^2 = 1.02$, d.f. = 1, $p = 0.31$; table 1). Moreover, pairwise comparisons showed that within-day variation in time spent singing DS significantly differed between long-encounter and short-encounter treatments ($\chi^2 = 18.6$, d.f. = 1, $p < 0.0001$), long-encounter and within-pair treatments ($\chi^2 = 18.3$, d.f. = 1, $p < 0.0001$), and short-encounter and within-pair treatments ($\chi^2 = 5.39$, d.f. = 1, $p = 0.02$).

Time spent singing US did not vary between morning and afternoon in the long-encounter treatment ($\chi^2 = 1.22$, d.f. = 1, $p = 0.27$), and the difference was marginally significant only in the within-pair treatment ($\chi^2 = 3.68$, d.f. = 1, $p = 0.06$). There was no interaction between time of day and treatment ($\chi^2 = 0.13$, d.f. = 1, $p = 0.72$; table 1).

Time spent singing AS was influenced by time of day in interaction with treatment (interaction time of day × treatment: $\chi^2 = 114.0$, d.f. = 2, $p < 0.0001$). Post hoc analyses revealed that time spent singing AS significantly decreased from morning to afternoon in the long-encounter treatment ($\chi^2 = 12.8$, d.f. = 1, $p < 0.001$), but not in the within-pair ($\chi^2 = 3.43$, d.f. = 1, $p = 0.06$), nor in the short-encounter treatments ($\chi^2 = 0.96$, d.f. = 1, $p = 0.33$; table 1). Moreover, pairwise comparisons showed that daily variation in time spent singing AS significantly differed between long-encounter and short-encounter treatments ($\chi^2 = 14.8$, d.f. = 1, $p < 0.001$), long-encounter and within-pair treatments ($\chi^2 = 17.2$, d.f. = 1, $p < 0.0001$), and short-encounter and within-pair treatments ($\chi^2 = 106.1$, d.f. = 1, $p < 0.0001$). Within-day variation in time spent singing across treatments is summed up in table 1.

(c) Consistency of time spent singing

All types of song were repeatable in the within-pair and the short-encounter treatments, whereas time spent singing US was repeatable only in the long-encounter treatment (table 1). However, time spent singing in the first trial was found to be

Table 1. Repeatability of and differences in the rate of each type of song (in seconds per minute) between morning and afternoon trials. All-song rate is computed from the sum of directed and undirected songs. Song rate differences between morning and afternoon trials are computed using Cohen's *d* with its 95% confidence interval (CI), and song rate repeatability is estimated using intra-class correlation coefficients (*R*) with their 95% CI and *p*-value. Values in bold indicate significant differences in song rate between morning and afternoon trials.

song type	treatment	morning rate (s min^{-1})	afternoon rate (s min^{-1})	Cohen's *d*	95% CI	*R*	95% CI	*p*-value
directed song rate	short encounters	7.45	6.28	−0.17	−0.44; 0.10	0.59	0.40; 0.77	<0.0001
	long encounters	**1.67**	**0.41**	**−1.37**	**−2.05; −0.69**	<0.01	−0.64; 0.66	0.48
	within-pair observations	0.32	0.23	−0.28	−0.58; 0.02	0.36	0.08; 0.64	<0.01
undirected song rate	short encounters	—	—	—	—	—	—	—
	long encounters	0.60	0.20	−0.35	−0.74; 0.04	0.59	0; 0.95	0.001
	within-pair observations	1.85	1.50	−0.29	−0.70; 0.12	0.29	0; 0.59	0.03
all-song rate	short encounters	7.45	6.28	−0.17	−0.44; 0.10	0.59	0.40; 0.77	<0.0001
	long encounters	**2.27**	**0.60**	**−1.16**	**−1.81; −0.51**	0.15	−0.49; 0.78	0.31
	within-pair observations	2.16	1.73	−0.31	−0.70; 0.08	0.31	0.02; 0.60	0.02

correlated to time spent singing in the second trial for each type of song in the long-encounter treatment (DS: $r_{10} = 0.73$, 95% CI [0.27;0.92], $p = 0.007$; US: $r_S = 0.60$, bootstrap 95% CI [0.02;0.92], $p = 0.002$, $n = 12$; AS: $r_{10} = 0.75$, 95% CI [0.30;0.92], $p = 0.005$).

(d) Influence of body condition on time spent singing

In all subsequent models, the three-way interaction between time of day, treatment and body condition was not significant ($\chi^2 < 2.57$, d.f. = 1, $p > 0.10$ for each model). In addition, the influence of body condition on time spent singing any type of song was never affected by time of day (interaction time of day × body condition: $\chi^2 < 0.26$, d.f. = 2, $p > 0.61$ for each model).

The influence of body condition on time spent singing DS differed between treatments (interaction body condition × treatment: $\chi^2 = 23.1$, d.f. = 2, $p < 0.0001$). Thus, there was a significant difference between within-pair and short-encounter treatments ($\chi^2 = 26.8$, d.f. = 1, $p < 0.0001$), but not between within-pair and long-encounter treatments ($\chi^2 = 1.46$, d.f. = 1, $p = 0.23$), nor between long- and short-encounter treatments ($\chi^2 = 0.02$, d.f. = 1, $p = 0.89$). By taking each treatment separately, body condition had a positive influence on time spent singing DS in the within-pair treatment ($\chi^2 = 9.10$, d.f. = 1, $p = 0.003$, $r_{78} = 0.30$), but had no effect in either the short-encounter ($\chi^2 = 0.08$, d.f. = 1, $p = 0.78$, $r_{100} = -0.01$) or the long-encounter treatment ($\chi^2 = 0.19$, d.f. = 1, $p = 0.67$, $r_S = 0.10$, $n = 24$).

The influence of body condition on time spent singing US differed between within-pair and long-encounter treatments ($\chi^2 = 13.9$, d.f. = 1, $p < 0.001$). Taken separately, body condition had a positive influence on time spent singing US in the within-pair treatment ($\chi^2 = 11.3$, d.f. = 1, $p < 0.001$, $r_{78} = 0.41$), but had no effect in the long-encounter treatment ($\chi^2 = 1.30$, d.f. = 1, $p = 0.25$, $r_S = -0.27$, $n = 24$).

The influence of body condition on time spent singing AS differed between treatments (interaction body condition × treatment: $\chi^2 = 22.4$, d.f. = 2, $p < 0.0001$). Thus, there was a significant difference between within-pair and short-encounter treatments ($\chi^2 = 32.2$, d.f. = 1, $p < 0.0001$), and between within-pair and long-encounter treatments ($\chi^2 = 4.42$, d.f. = 1, $p = 0.036$), but not between short- and long-encounter treatments ($\chi^2 = 0.18$, d.f. = 1, $p = 0.67$). By taking each treatment separately, body condition had a positive influence on time spent singing AS in the within-pair treatment ($\chi^2 = 12.4$, d.f. = 1, $p < 0.001$, $r_{78} = 0.43$; figure 2*a*), but had no effect either in the short-encounter treatment ($\chi^2 = 0.08$, d.f. = 1, $p = 0.78$, $r_{100} = -0.01$; figure 2*b*) or in the long-encounter treatment ($\chi^2 = 0.20$, d.f. = 1, $p = 0.65$, $r_S = -0.02$, $n = 24$; figure 2*c*). Paired males in good condition sang at a higher rate than paired males in poor condition.

Six pairs out of 40 did not show any affiliative behaviour during the experiment. Removing the corresponding males from the analyses did not change the significant influence of body condition on time spent singing in the within-pair treatment (DS: $\chi^2 = 9.13$, d.f. = 1, $p = 0.003$, $r_{66} = 0.39$; US: $\chi^2 = 10.6$, d.f. = 1, $p = 0.001$, $r_{66} = 0.43$; AS: $\chi^2 = 12.1$, d.f. = 1, $p < 0.001$, $r_{66} = 0.46$).

The influence of body condition on time spent singing did not vary according to male status (preferred versus non-preferred) in the within-pair treatment (interaction male status × body condition: DS: $\chi^2 = 2.73$, d.f. = 1, $p = 0.10$; US: $\chi^2 = 0.50$, d.f. = 1, $p = 0.48$; AS: $\chi^2 = 0.99$, d.f. = 1, $p = 0.32$).

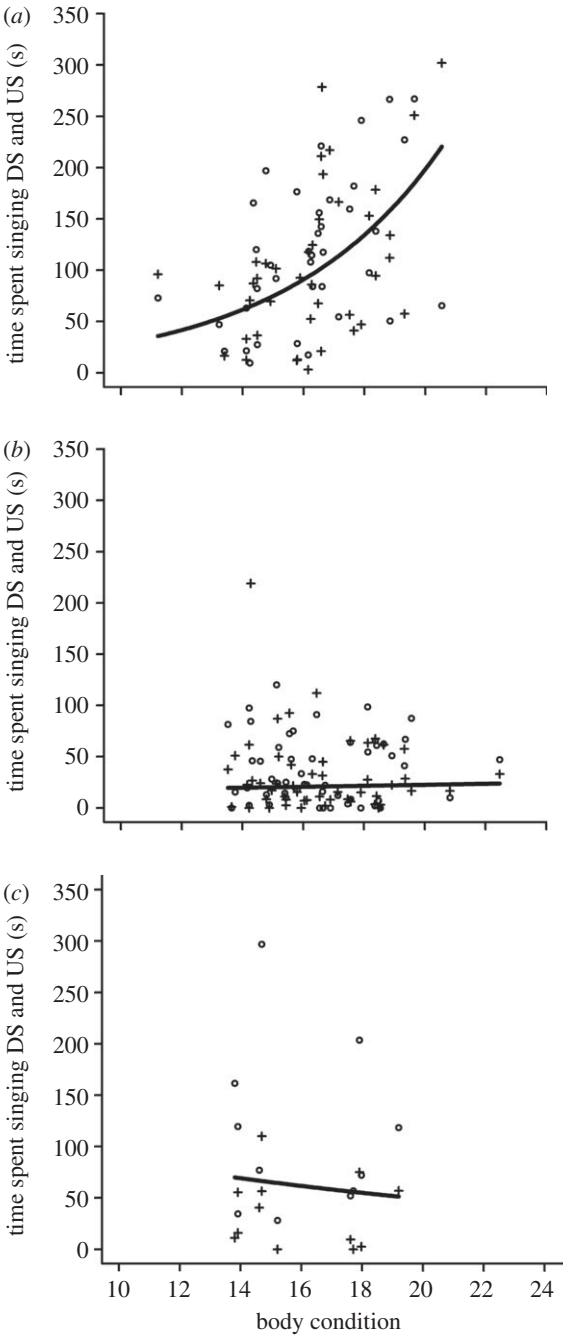

Figure 2. Influence of body condition (computed as the scaled mass index) on time spent singing both directed songs (DS) and undirected songs (US) (*a*) within mated pairs (2 × 1 h recording), (*b*) during short-encounter trials (2 × 5 min recording) and (*c*) during long-encounter trials (2 × 1 h recording). Each male is represented by two points, corresponding to the morning (circles) and the afternoon trials (plus symbols).

4. Discussion

(a) Behavioural consistency

Results from this study indicate that condition-dependence of male song rate can be influenced by pairing context in zebra finches. Time spent singing was found to be a reliable signal of male condition in a pair-bond context, but not during encounters between unfamiliar individuals. These results suggest that time spent singing is not a reliable signal of quality in all situations, at least in zebra finches, but that male condition assessed via song can play a role in female mating decisions after the pairing process [36].

We have demonstrated that time spent singing both DS and US song rate were overall repeatable for within-pair observations and short-encounter trials, indicating that males differ from each other in their song production in a sexual context. The repeatability estimates of time spent singing for within-pair observations were similar to the ones computed in a recent meta-analysis ($R \sim 0.40$) under the heading 'courtship' [53]. However, our estimate of time spent singing repeatability for short-encounter trials was higher than 0.40. The heading 'courtship' in Bell *et al.*'s [53] meta-analysis is composed of several different behaviours other than song rate, such as body movements, collected from different species and taxa. For this reason, it is difficult (maybe impossible) to determine what causes the discrepancy between the two estimates. The amount of directed singing produced during long-encounter trials was not repeatable in our study. However, this has to be contrasted with correlation analyses, which showed that time spent singing in the second trial was correlated with time spent singing in the first trial. As 'long encounters' is the only treatment for which time spent singing significantly decreased between morning and afternoon, it is conceivable that the lack of repeatability obtained through ANOVA-based analyses is due to high intra-individual variation between trials. Several reasons could also account for the observed lack of repeatability: first, long-encounter trials have been performed in a different captive colony than short encounters and within-pair observations. Repeatability of behaviour has already been found to vary between populations [54,55]. It is thus possible that individuals from the two different colonies used in this study differ in their level of behavioural consistency [56]. We believe that this possibility is worth investigating, as differences in individual consistency, or intra-individual variability, may have fitness consequences [57,58].

Second, the lack of repeatability during long encounters may be the mere outcome of the design used in this treatment in comparison with the two others. Indeed, a sexual encounter is a very stimulating situation for a male zebra finch for several reasons. First, both sexes have been kept most of the time separated from each other in the two captive colonies used here. Second, evidence from previous studies conducted with zebra finches shows that a female's decision to copulate with a newly met male occurs within the first few minutes of an encounter [33,59]. However, first short-encounter trials, in contrast to long ones, might have been too brief (5 min) for females to fully assess male quality and to make the decision whether to copulate or not. Therefore, male willingness to display during the second trial, even in front of the same female, may have remained unchanged, leading to repeatable estimates of time spent singing across trials. By contrast, it is conceivable that the first 1 h long-encounter trial was long enough to settle the interaction, and for females to assess males. This in turn may have reduced, or at least affected, male willingness to display during the second long-encounter trial.

(b) Body condition and song rate

Our results show that the link between condition and time spent singing depends on the situation within which males are displaying in front of females. Only paired males in good condition sang at a higher rate than poor-condition paired males. No such difference was observed for unpaired males either during short or long encounters. The possibility remains that body condition does not have a direct influence on time spent singing but that the covariation of the two variables is under the influence of a third one that varies between situations. Experimental alterations of male condition would be needed to determine the causal nature of the relationship between body condition and time spent singing. Still, our findings can have implications for the study of signal use in sexual selection. First, investigating the link between song rate (and ornaments in general) and body condition at different stages during pair formation, and more generally in different contexts, might be of importance [15,60]. It is possible that condition may not be a good proxy for quality in different situations or different contexts [61]. Previous studies [29,30] may have failed to detect a link between body condition and song rate because male behaviour was recorded only during the first few minutes of interactions between mates [24], a situation where male singing activity may reflect only sexual motivation. Second, long-encounter trials lasted the same period of time as within-pair observations. This experimental design enabled us to distinguish the effect of time and pairing context on the relationship between body condition and time spent singing. Time spent singing was found to be unrelated to body condition during long-encounter trials. Thus, it appears that the limited time available to males to display during short-encounter trials cannot explain the failure to find a significant relationship between body condition and time spent singing. Evidence then suggests that male pairing context is a better predictor of song-rate condition-dependence than time available to display. Differences in condition-dependence between treatments may lie in the fact that male motivation to display was higher during encounter trials [62]. Contrary to paired males, access to females was limited for single males so that they may have devoted as much time and energy into displays as they possibly could [38]. Our results indicate that even poor-condition males can sustain a high rate of singing when trying to obtain copulations. Time spent singing thus appears to be an unreliable signal of body condition of unfamiliar males (at least over the first hour of an interaction with an unpaired female). By contrast, our findings suggest that paired females may reliably assess their mate's condition through the energy and time devoted into singing. These hypotheses may be related to zebra finches' ecology. These birds are colonial both during breeding and non-breeding seasons, during which they form feeding flocks of up to 20 and 350 individuals, respectively [31]. Moreover, they nest in colonies of up to 50 nests [31]. Zebra finches' coloniality thus offers the opportunity for females to gain information on potential mates or extra-pair mates, insofar as they can devote enough time to assess their singing activity. However, further studies would be needed to determine to what extent females can really use such information, given flock stability or the amount of attention they can allocate to the assessment of the quality of extra-pair mates [63]. Finally, the fitness consequences of pairing with good- or poor-condition males also deserve further investigation [26].

(c) Signal use in a monogamous context

Our results may have implications for the study of female preference in a monogamous context. This particular mating system provides females with the opportunity to continuously assess their partners' quality [64,65]. This differs, for instance, from lek systems, where females have a very limited period of time during the reproductive season to assess potential mates and get inseminated. In accordance with the present findings, a recent model has shown that

long-term commitment, such as within monogamous pair-bonds, can promote honest signalling [38]. Although this model was about aggressive communication, we think that it could be applicable to the present context, as a pair represents a long-term commitment, with important consequences on individual fitness. Yet the difference between this model and our findings is that the former does not imply any differential costs of signalling between different individuals. Conversely, in accordance with the handicap hypothesis [5], we assert that poor-condition birds cannot afford to sustain a high rate of singing in a pair context, due to related costs. Some studies show that female birds can modulate their preference for their social partner over the course of the pair-bond [66,67], eventually leading to mate-switching [68]. Our study suggests that time spent singing could function as an honest signal of male body condition that females could use to assess their own mates' qualities, at least in zebra finches. Conversely, females could not easily infer male condition from their singing activity during brief mate sampling as even individuals in poor condition seem able to sing at a high rate under such circumstances. In accordance with these hypotheses, some evidence exists that female zebra finches pay more attention to the songs of their mates [35] than to the song of unfamiliar males ([32,33] but see [69]). Further evidence is needed to determine the influence of a mate's singing activity on female likelihood to prospect for other potential mates, search for extra-pair copulations or divorce.

We propose that future studies should more systematically take species characteristics, such as mating systems, into account to generate relevant hypotheses about the evolution of the condition-dependence of sexual ornaments.

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